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DEVELOPMENT OF CHRONIC INSERTABLE OXYGEN ELECTRODES. (U)

OCT 76 R E BARR, A W HAHN

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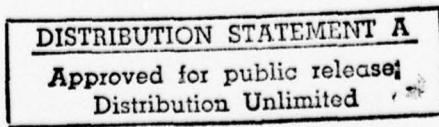
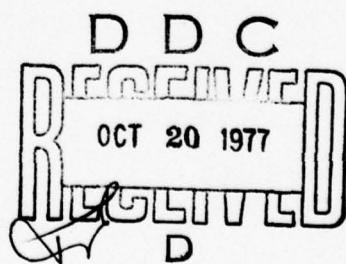
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ANNUAL REPORT

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"The Development of Chronic Insertable
Oxygen Electrodes"

Contract No. DADA 17-71-C-1104



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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Work has been in progress towards developing oxygen sensing electrodes with long-term stability characteristics. There are two types of changes that can occur with oxygen electrode responses. One is noted by a comparatively rapid decrease in current output while the other is termed a slow drift of decreasing value in electrode current. The latter phenomenon has been called electrode "aging" and it is toward the solution of this problem that a great deal of effort has been directed. A technique of periodically anodizing O ₂ sensing electrodes has been → over		

20. Abstract

developed that appears to significantly delay or eliminate the slow downward drift in current. Stable electrode currents for periods of three days have been obtained. This periodic anodization seems to be effective only on electrodes whose surfaces have been activated by an anodization protocol. The process appears to work on bare tipped as well as membrane coated electrodes.

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ANNUAL REPORT

"The Development of Chronic Insertable Oxygen Electrodes"

Ronald E. Barr

Allen W. Hahn

October 1976

Supported by U.S. Army Medical Research

and

Development Command

Washington, D.C. 20314

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University of Missouri

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Contract Period; The period covered by this report is from 1 August 1975 to 31 October, 1976.

Contract Objectives:

For the contract year 1975-1976, the objective of the program was to develop stable, insertable or implantable oxygen electrodes for use in soft tissue. Work towards this objective proceeded in a number of areas: (1) Control of electrode "aging", (2) electrode membrane studies, (3) implantable electrode system and (4) a telemetry system for transmitting oxygen current data. In preparing the proposal for continuation of the project, a new area of application was considered - vascular blood gas monitoring. Efforts made and findings obtained in each of these areas will be presented below.

1. Control of Electrode Aging. One of the chief causes of electrode drift is the "aging" phenomenon associated with changes in the electrode surface characteristics caused by the interaction of oxygen with the metal atoms of the electrode. Using bare-tipped electrodes in sterile saline, a method has been developed to control the aging process. Details of early results of this work were presented in annual reports for 1974 and 1975. Continuing efforts have shown the simplest method of controlling electrode aging is the initial anodization of the electrode at a voltage above the oxygen evolution potential (ca., 1.25 V vs Ag/AgCl) prior to calibration and usage, followed by periodic anodization during use. In early tests, each electrode had been anodized initially for 30 minutes followed by 5 minute anodization periods after every 85

minutes of cathodization. More recently, attempts have been made to reduce the anodization time and the recovery time during the first part of the cathodization cycle. Fig. 1 shows results of recent studies. In part A, different sized electrodes were anodized for one second. The curves show the rate of recovery for each electrode. In part B, 25 um electrodes were subjected to different anodization times. The curves show the rate of recovery following recathodization.

The technique of periodic anodization has been applied to bare and coated electrodes in saline, artificial biological fluid (ABF) and blood plasma (horse). The technique seems to be applicable in all of these conditions, Table 1. However, the analysis of the effects of periodic anodization on electrodes in ABF and blood plasma was and is

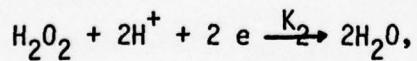
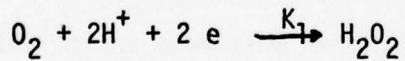
Table 1. Average cathodic current drop of five 15 um bare electrodes after 15 hours of continuous polarization in three different fluids. Values in percent.

	Saline	ABF	Plasma
without anodization	10.6	13.4	12
with anodization	4.3	1.2	*

*Insufficient amount of data to draw meaningful values.

confounded by the "poisoning" phenomenon that occurs in these types of media. A partial solution of separating the aging and poisoning effects on electrode current is by monitoring current changes when stirring the solution. Stirring affects the poisoning phenomenon by washing the electrode surface and, if aging has not influenced the current level, the current will be at a level at least equal to its initial value immediately after stirring has ceased. At present this method of separating aging and poisoning effects is only qualitative.

It has also been found that new or freshly polished electrode surfaces are more unstable than previously used electrodes and that an initial anodization of the new electrodes enhances initial stability. These findings are illustrated in Fig. 2 and Fig. 3. The reason why this procedure works is that the anodization oxidizes the Pt surface which has the following effect: The complete reduction of oxygen to water is a two stage process:



where K_1 , and K_2 are the specific rate constants per unit area for the respective reactions. On oxidized Pt (PtO), K_2 is about an order of magnitude greater than K_1 , whereas on reduced Pt (freshly polished surface), $K_1 > K_2$. Thus on oxidized Pt, all of the H_2O_2 that is formed by the first reaction is reduced to H_2O , whereas on a freshly

polished Pt surface, not all of the H_2O_2 produced by the first reaction is reduced to H_2O ; some of it diffuses into the solution.

Electrode current stability may be described in these terms by considering the interpretation given to the graphs in Fig. 2 and Fig. 3. Fresh surfaced electrode current (Fig. 2) begins at a low value, approximately one-third to one-half of the theoretical maximum value, increases during the first few hours, peaks and then decays asymptotically to some value near one-third to one-fourth theoretical maximum, after two to three days operation. An interpretation explaining part of this graph is as follows: Since the electrode surface consists of reduced Pt, the

two electron reaction takes place to produce H_2O_2 . Initially there is a very steep concentration gradient for H_2O_2 to diffuse into the bulk solution. This process competes with the second two electron reaction. However, following cathodization there is a build up of H_2O_2 concentration surrounding the electrode and, as the H_2O_2 concentration increases, more and more H_2O_2 is reduced to H_2O (second reaction). Thus the current increases. After some time the H_2O_2 concentration gradient reaches an equilibrium state, thereafter, the amount of H_2O_2 reduced remains constant for a given electrode surface condition.

It is obvious from Fig. 2 that electrode current does not remain constant, but rather, begins to decrease. This is due to electrode surface "aging", a phenomenon we do not fully understand but one that reduces the number of catalytic sites per unit area of electrode surface. This decrease in active sites affects both reactions of the oxygen reduction process, causing a reduction in electrode current.

On the other hand, electrode surfaces that have been initially anodized have part of that surface converted to PtO. If the anodization process was long enough, then enough PtO is present so that all O_2 molecules reduced at the surface lead to a four electron reaction, thus yielding the maximum theoretical current. This is because both reactions take place, since for PtO, $K_2 \gg K_1$. Therefore, as shown in Fig. 3, the initial current is high. The PtO is reduced to Pt during cathodization within the first couple of hours of operation. Meanwhile, there has been a delayed buildup of H_2O_2 near the surface, since most of it has been reduced to H_2O . This causes a delay in the current peak corresponding to H_2O_2 buildup, as described above. Thus in Fig. 3 this peak is indicated to be at about four hours whereas in Fig. 2 it occurred at about two hours. As with non-anodized electrodes, there is a continuous reduction in the catalytic activity of the Pt surface with time, causing electrode current to continuously decrease. For some as yet unexplained reason, the rate of current decrease beyond 10 hours for electrodes that had been anodically pretreated has been less than for untreated electrodes.

The periodic anodization of electrodes has been found to be effective only when applied after about five to ten hours cathodization. Periodic anodization prior to this time disturbs the establishment of the quasi-steady state H_2O_2 concentration gradient and, therefore, causes large changes in electrode current. This would be unacceptable in practice.

Studies on the problem of aging are continuing. The descriptive model presented above represents an initial attempt to understand what is happening in the "aging" process. Further quantification and testing of this model is needed to ascertain its usefulness.

2. Electrode Membranes. Membranes on oxygen electrodes have traditionally been used to prevent or retard "poisoning" of the electrode surface by protein and other biological molecules that are electrolytically attracted to the surface. This indeed is one of the important uses of a membrane. Even so, membrane covered electrodes, especially the most commonly used Clark type electrode, are subject to long term current drift. This drift is only partly due to the aging process that occurs with or without a membrane. It is also partly due to the other design considerations that will not be elaborated upon in this report (See previous reports and proposals). Because of these design problems our project has focused on electrodes that have only the recording electrode covered by a membrane. The reference electrode is placed external to the membrane. For a current to flow between the two electrodes, the membrane must be hydrophilic and permeable to oxygen.

This project has focused on cold plasma deposited polymer membranes, chiefly using the monomer chlorotrifluoroethylene (CTFE). Some work have has been performed with liquid dip membranes, primarily polystyrol.

Considerable effort has been put into trying to develop a CTFE membrane that would rigidly adhere to a glass insulated electrode surface

and that would demonstrate the characteristics required of an electrode membrane. To date these efforts have not met with the kind of success required for potential use in applied work. The chief problem has been lack of adhesion between the glass insulation and the CTFE membrane.

Two avenues of investigation have been followed in attempting to skirt this problem. First, an intermediate membrane, polystyrol, has been introduced that adheres well to glass and to which CTFE adheres. With this preparation, one obtains tenacious membranes, but we have found increased variability in electrode current responses. It is thought, but not proven, that some of this variability may be due to the type of polytyrol membrane that has been produced. After a number of discouraging trials, a scanning electron microscopic investigation of the polystyrol membranes was undertaken. The type of membranes observed is indicated in Fig. 4. This is a scanning electron micrograph of a polystyrol membrane which was put on an electrode surface. The important observation is the pits in the membrane. It is hypothesized that these are due to the manner in which the solvent evaporates. Each time a polystyrol membrane is formed, the evaporation process is slightly different, which could be leading to different electrode responses. For reference, the light region under the membrane in the center of the photograph is 25 um electrode wire. The cracks in the membrane were caused in preparing the sample for SEM viewing.

The second approach has been to try to find another type of insulation to which the CTFE membrane will rigidly adhere. To date, work

has concentrated on various epoxy resins, and the product HYSOL (T.M.R. Materials Co., Kansas City) has yielded satisfactory characteristics. That is, HYSOL resin bonds securely to the electrode wire, is hard enough to permit electrode surface polishing and CTFE and polystyrol adhere tenaciously to it.

Using HYSOL as the insulation material, some very encouraging results have been obtained for long term operation of electrodes in saline. Fig. 5 shows the response of two electrodes coated with CTFE and Fig. 6 shows two electrodes coated with polystyrol. For both types of coatings there is a period over which electrode currents are below their oxygen diffusion limited values. We postulate that during this time, the membranes are hydrating and the current is not oxygen diffusion limited. Why some electrode membranes cause currents to be at very low values and then suddenly increase to oxygen diffusion limited values, as shown by three of the four currents in Figs. 5 and 6, is not clear at this time. Note that after the first -- hrs of operation, 1 second anodization pulses were applied every three hours.

3. Implantable Electrode System. During the course of this program, an in vivo implant electrode package consisting of four oxygen electrodes, tie-down collars, lead wires and a transcutaneous connector device was designed and tested in rabbits. Only minor modifications to that shown in Fig. 9 of the 1973-74 Annual Report have been made. The electrodes were implanted in longissimus muscle of rabbits and the transcutaneous device was mounted over the sacrum. With this set-up, oxygen currents were recorded up to 80 days post-implantation.

From a practical viewpoint the data obtained was not very useful. Relatively consistent and noise free data could be obtained only when the animal was anesthetized, Fig. 7. In the unanesthetized state, biological electrical noise and physiological control mechanisms totally obliterated achieving useful data, Fig. 8. Aside from this difficulty, histological examination of tissues surrounding electrode sites revealed that a mild chronic inflammatory response was continuously present, Fig. 9. It has become clear that using this type of oxygen electrode implanted in a site where there is apparently considerable vascular flow control yields highly variable data. The practical applicability of this type of system to potential clinical problems is highly questionable. For this reason this part of the project has been indefinitely discontinued.

4. Telemetry System. A telemetry system is a convenient system for achieving oxygen tension data from chronically implanted electrodes. It is the only practical system to use in unanesthetized unrestrained animals. During the past year a single channel short range (~20 feet) telemetry system was designed, constructed and tested. Details of the transmitter and receiver circuitry along with sample data are presented in the accompanying paper that was presented at the 3rd International Symposium on Biotelemetry, May, 1976 at Asilomar, California. Presently, work is proceeding on the development of a four channel multiplexed telemetry system. It has undergone initial testing and is in the process of being modified.

Other Developments:

Blood Catheter Electrodes. Due to the impasse reached in tissue implant studies and because of the needs of the contracting agency, our program has shifted to development of a blood gas catheter system. The emphasis of the proposal for 1976-77 was on this system. Since the submission of the proposal and in conjunction with the four channel telemetry system mentioned above, a four oxygen electrode catheter package has been built and is being tested in vitro presently. With this system, the plan is to obtain knowledge of technical problems that will be encountered in developing and applying blood gas catheter electrodes for chronic implantation. The package has been designed to contain one arterial electrode, one venous electrode and two tissue electrodes. They will be implanted through the carotid artery, the jugular vein and subcutaneously, respectively, in a dog.

Personnel Changes. There has been changes in the personnel associated with the program during the past year. Dr. V. G. Murphy left in September, 1975 and was replaced by Dr. Thomas Tang, who joined the project in December, 1975. Dr. Tang is a chemical engineer and did his M.S. and Ph.D. degree work at the University of Iowa. The subject of his doctoral dissertation was "Mass Transfer of Dissolved Gases Through Membrane Tubings". Our technical assistant, Mrs. B. MacAlmon resigned to have a baby in March. She was replaced by Mr. Patrick Quigley, a National Merit Scholar who attended the University of Missouri for two years.

Summary:

In summary, during the past contract year, the program has evolved a qualitative model to explain a number of aspects of electrode response characteristics. The periodic anodization protocol has been optimized. A number of changes were made to improve the operating characteristics of membrane coated electrodes. The in vivo muscle implant oxygen electrode project reached a point where decisions concerning the potential future applicability of the system could be made. A single channel telemeter system was built and tested for recording tissue oxygen tension data from unanesthetized unrestrained animals. A four channel system is currently under test. Finally, the projects applications goals have been shifted to emphasize blood gas monitoring.

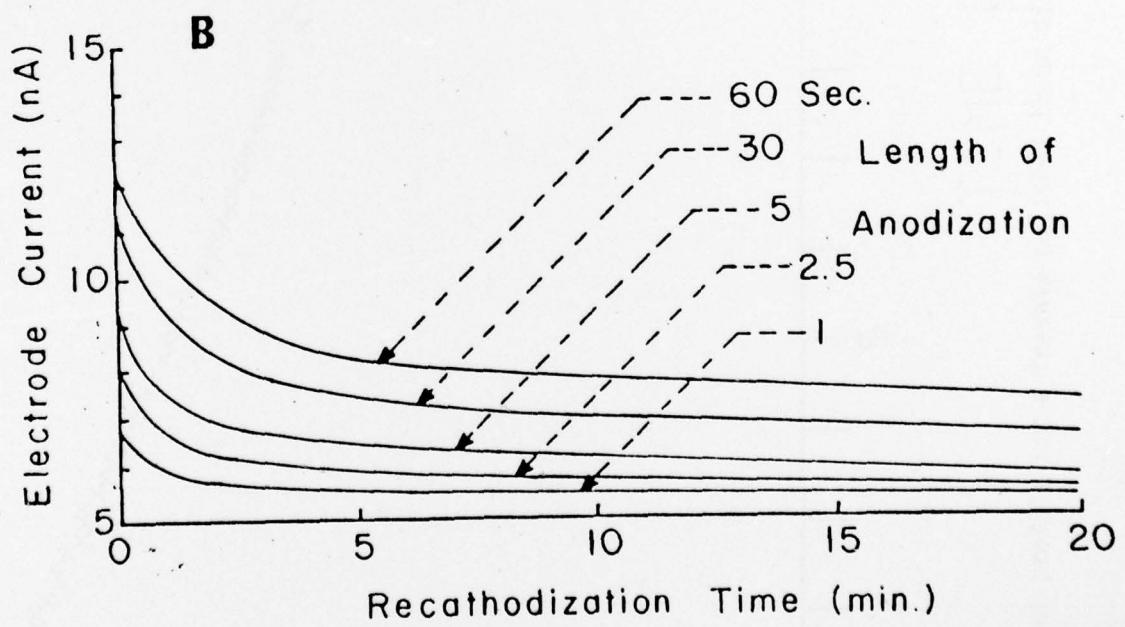
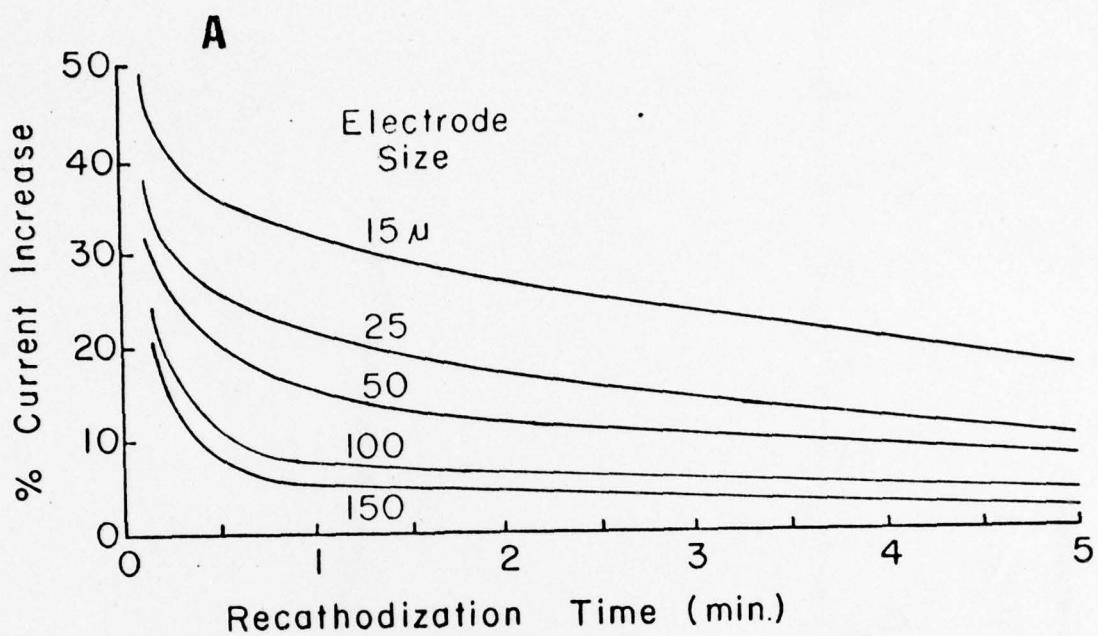


Fig. 1. Recovery rates of electrodes following anodization. See the text for a full description.

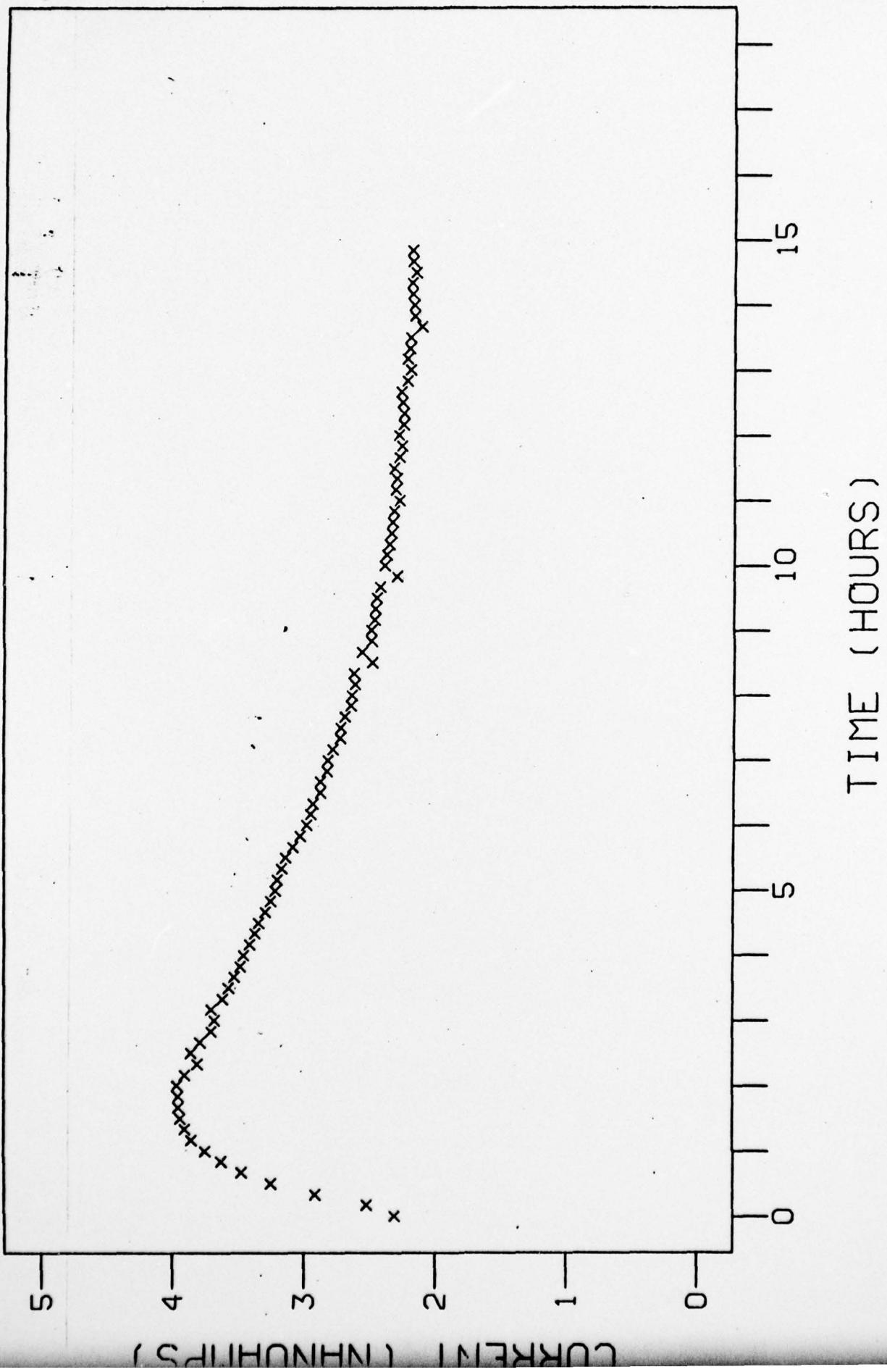


Fig. 2. Current profile for a freshly polished 15 μm diameter electrode.

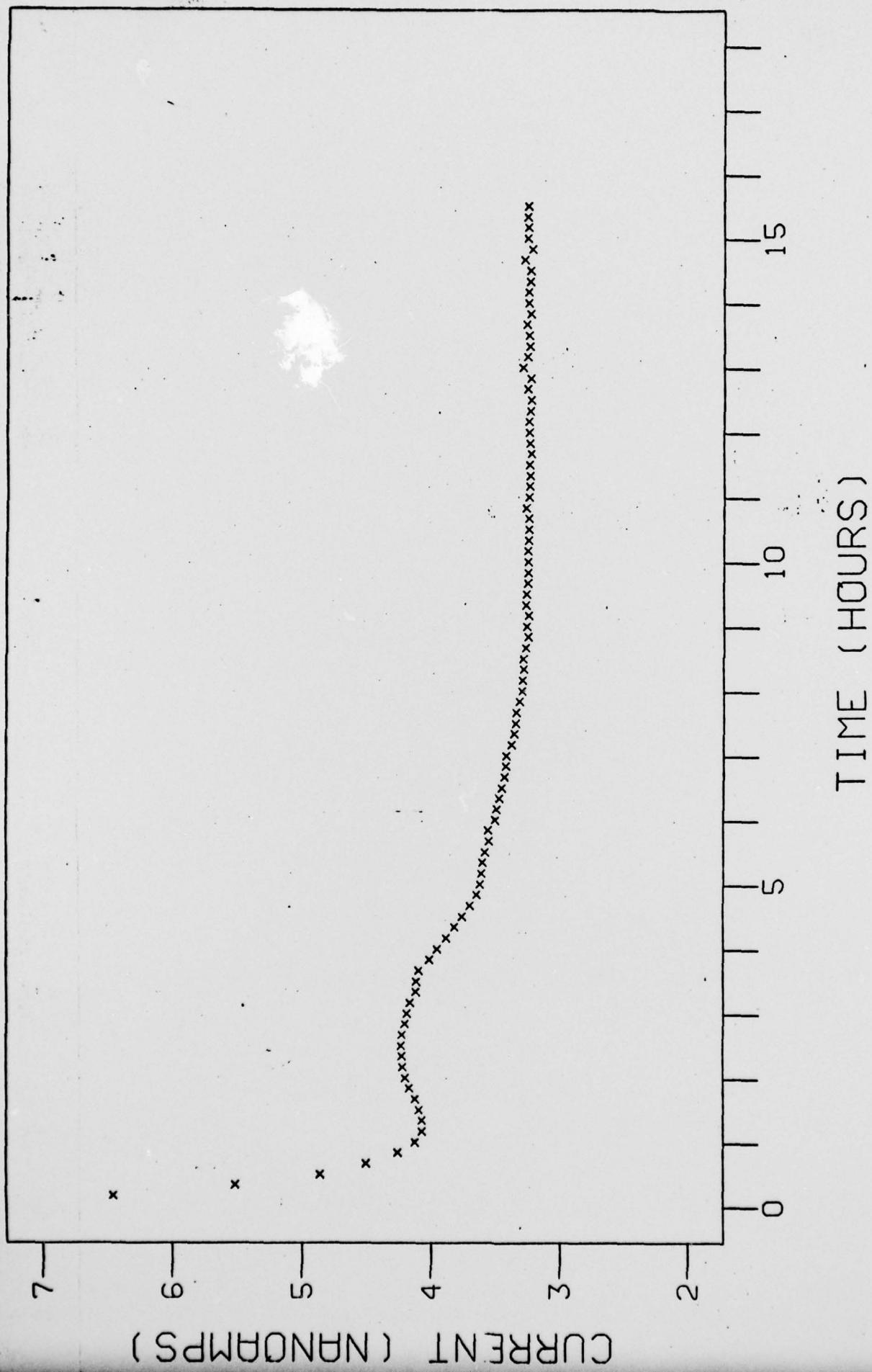


Fig. 3. Current profile for a freshly polished 15 um diameter electrode following anodization.

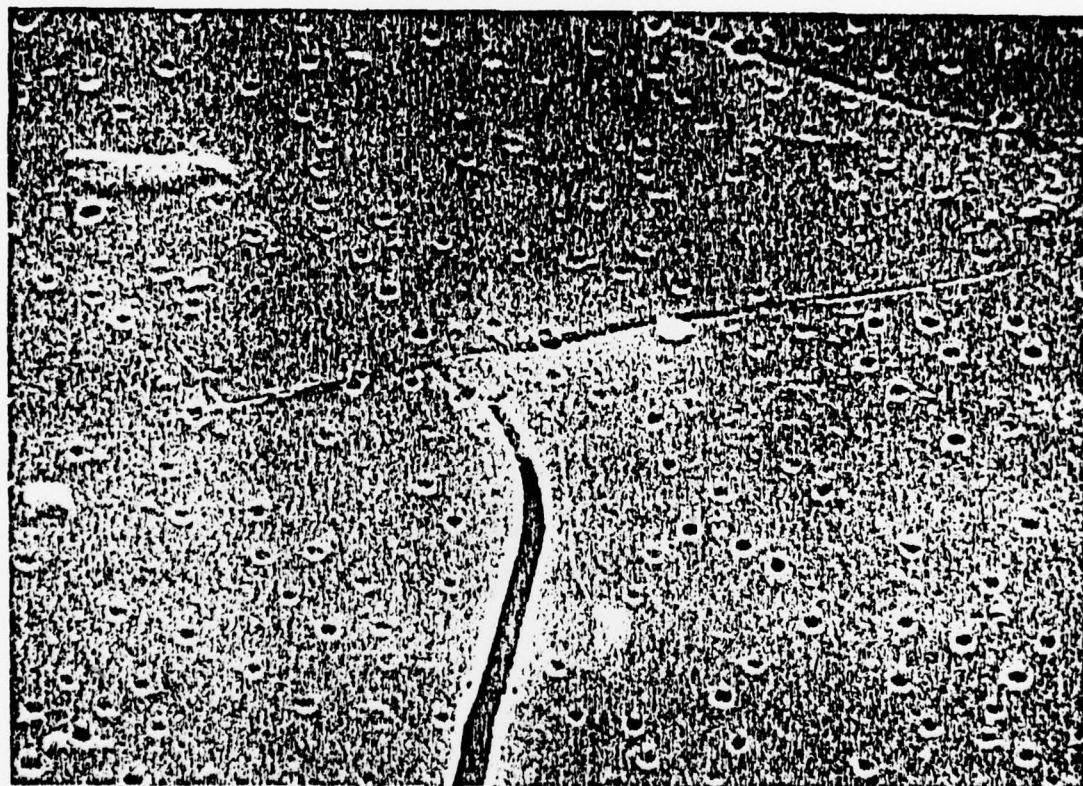


Fig.4. A scanning electron micrograph view of a polystyrol coated electrode. See the text for further description.

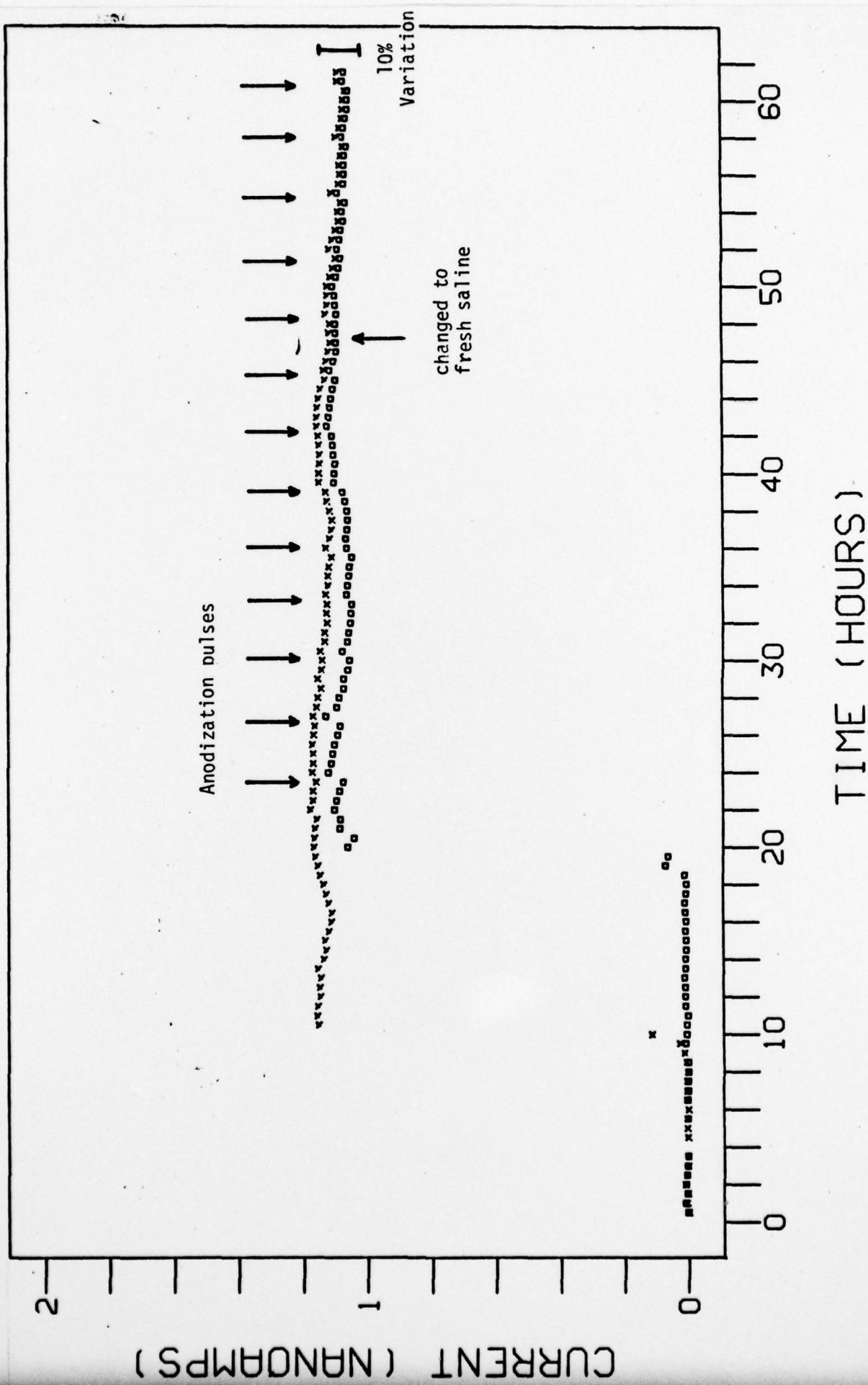


Fig. 5. Current profile of two CTFE coated electrodes subjected to one second anodizations every three hours following current buildup.

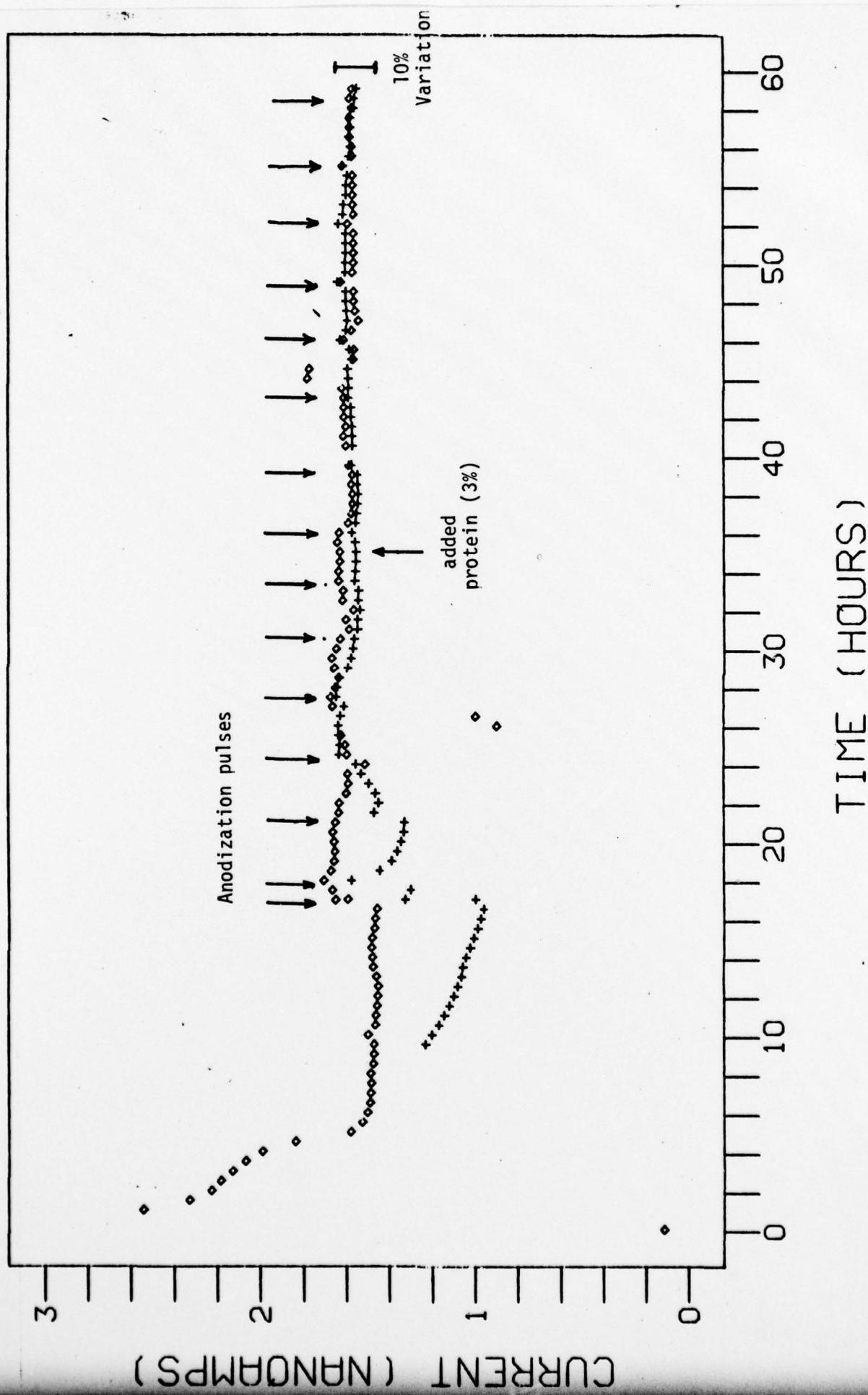


Fig. 6. Current profile of two polystyrol coated electrodes subjected to one second anodizations every three hours starting at hour 15.

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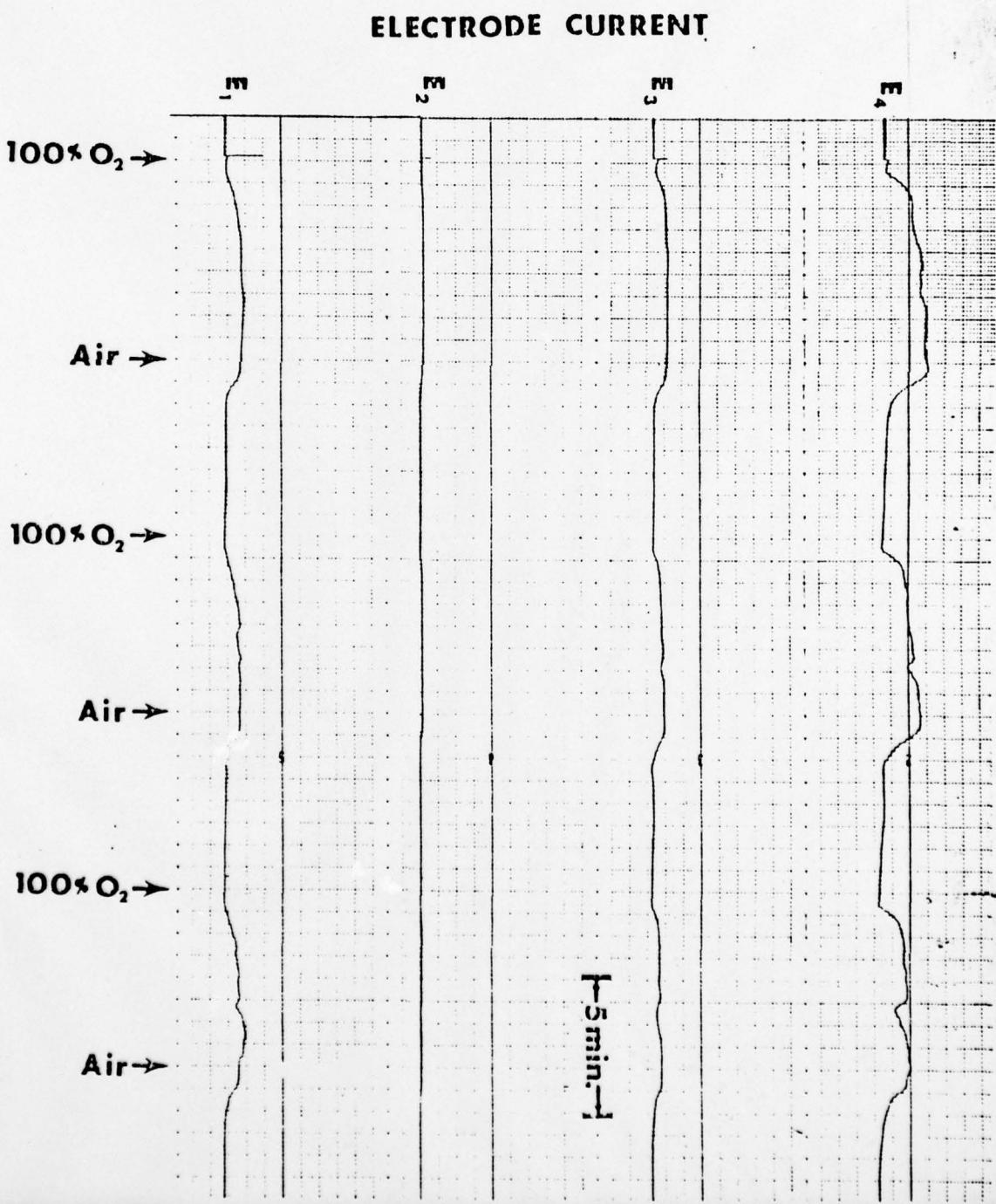


Fig. 7. Response of four oxygen electrodes implanted in rabbit muscle to changes in respiratory gas in an anesthetized animals. Time progresses from left to right.

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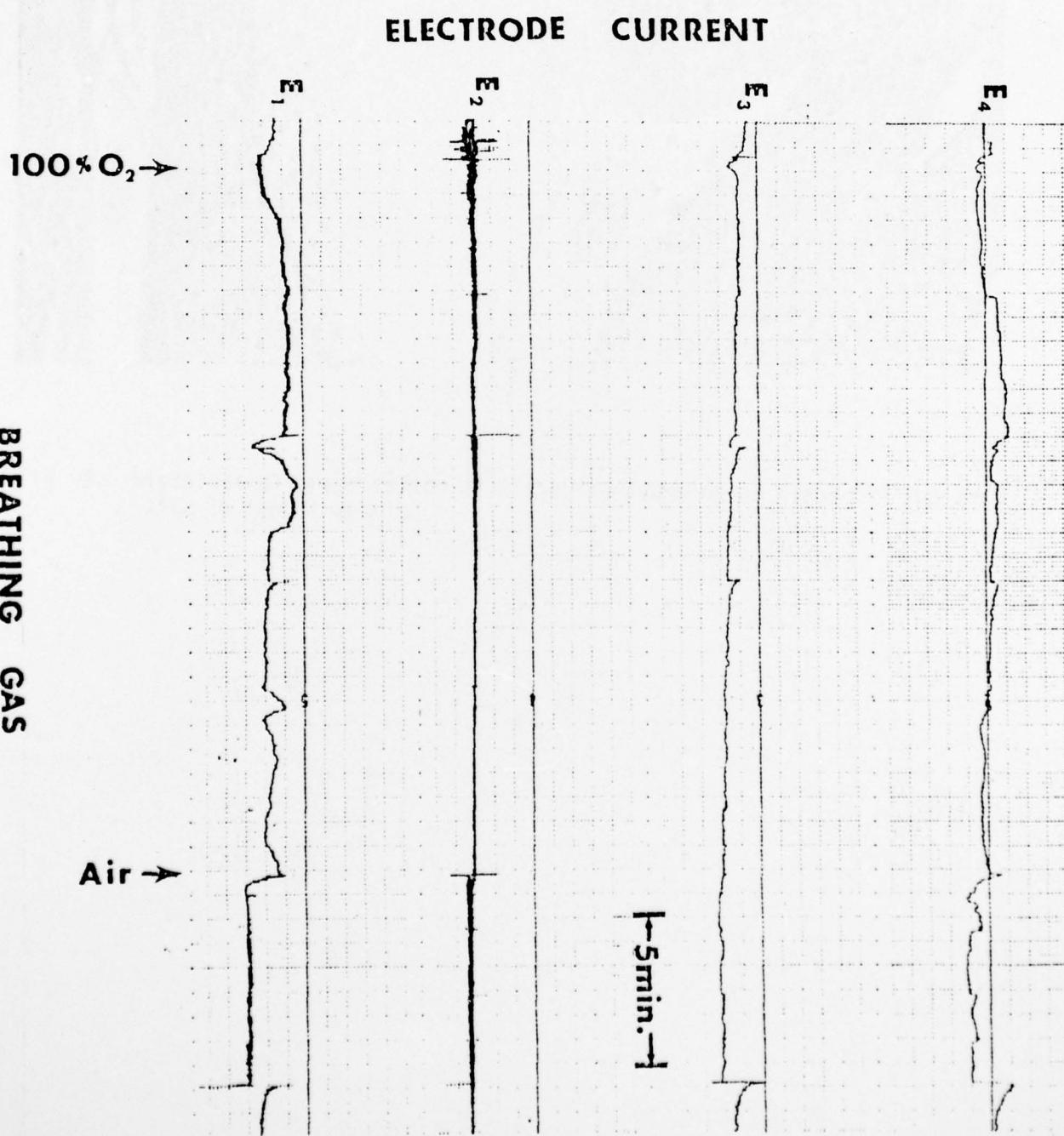


Fig. 8. Response of four oxygen electrodes implanted in rabbit muscle to changes in respiratory gas in an unanesthetized rabbit. Time progresses from left to right.



Fig. 9. Histological presentation of tissue surrounding an electrode implant site in rabbit muscle.

SHORT RANGE TELEMETRY OF TISSUE OXYGEN TENSIONS

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Monitoring of tissue or blood oxygen partial pressure (P_{O_2}) is important in a number of areas of clinical and research medicine (1). Telemetry provides a convenient, if not necessary, means for continuous measurement of tissue and blood P_{O_2} for long term (2-5 days) studies. Modern integrated circuit technology has made available MOSFET input operational amplifiers where high input impedance, low bias current, and low power drain make them well suited to the monitoring of the nanoampere O_2 electrode currents in a miniaturized P_{O_2} telemetry system. This paper describes our initial efforts to develop such a system.

METHODS

The transmitter (Fig. 1A) consisted of a current-to-voltage converter with a buffer amplifier driving a voltage controlled oscillator (VCO) to produce a subcarrier for the single transistor frequency modulated (FM) transmitter. The current-to-voltage converter was a single RCA CA3130 FET-input integrated circuit that was followed by a second CA3130 connected as an inverting amplifier to provide both gain and offset control. The signal voltage was input to the VCO section of a RCA CD4046 to generate a frequency of 200 Hz for zero current. The linear range of the VCO extended to approximately 9 KHz, which corresponded to an input current of 18 nanoamperes. The signal was used to swing the frequency of the single transistor oscillator. The fundamental of the transmitter oscillator was approximately 20 MHz but harmonics could be received by broadcast band FM receivers.

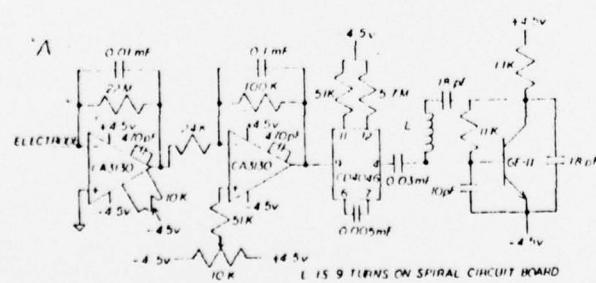


Fig. 1. A. Circuit diagram of the transmitter.

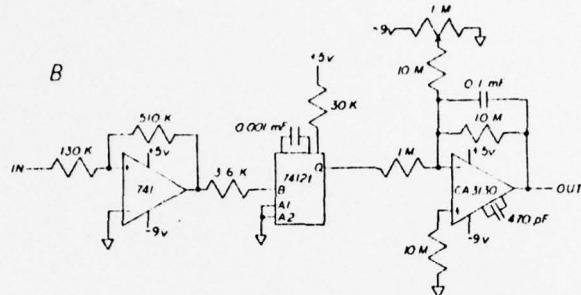


Fig. 1. B. Circuit diagram of the receiver subsystem.

The receiver subsystem (Fig. 1B) consisted of a standard FM receiver equipped with AFC and AGC to demodulate the FM subcarrier. The receiver audio-output was passed through a 4-pole active band-pass filter, and into a comparator with a 2 volt hysteresis. The comparator output was used to trigger a 74121 monostable multivibrator with a 10 μ sec. pulse width. The multivibrator output was integrated using a RCA 3130 operational amplifier with a one second integrating time constant in the feedback network. The integrator output, proportional to the audio frequency, was recorded on the strip-chart recorder.

For the purposes of testing the system, bare tipped, 25 micrometer diameter platinum (insulated in glass) electrodes were fabricated into an implant package. The implant package consisted of four electrodes, tie down collars, lead wires and a transcutaneous connector device. Details of this package, the surgical implantation procedure and their mode of operation were given earlier (2). After approximately one week post-surgery, after implanting the electrodes, changes in inspired P_{O_2} produced changes in electrode currents indicating changes in the surrounding tissue oxygenation. The transmitter and batteries were placed in the pockets of a vest worn by the rabbit. To obtain good illustrative data, the rabbit was anesthetized with halothane, although data from unanesthetized restrained and unrestrained states were also collected.

RESULTS

A calibration curve of the telemetry system, in which output voltage is plotted as a function of input current, is shown in Fig. 2. The response was linear over a range from approximately 5 to 15 nanoamperes input current. At lower currents the response was nonlinear and less sensitive.

Electrode current is dependent on oxygen, polarization voltage, motion artifact, "aging" and "poisoning" phenomena. The present investigation was not concerned with absolute oxygen tension values but only with relative changes. Hence, the polarization voltage was set for a given run and the system was operated

until the electrode current had stabilized before a forcing gas was applied. For short runs, the aging characteristic was unimportant and motion artifact, if it existed, was a small perturbation to the data.

A sample of data obtained is shown in Fig. 3. It can be seen that the output voltage of the telemetry system, curve A, closely matches the shape of the input current, curve B. For the sample shown, the lower valued portions of curve A correspond to the relatively insensitive portion of the telemetry system's response curve and, therefore, is not as responsive to changes in the input current at these levels.

DISCUSSION

Continuous monitoring of tissue or blood oxygen tension for a period of days has many problems associated with it.

One of these problems is that it would be impractical to attempt to collect such data by "hard-wire" methods. Even short term

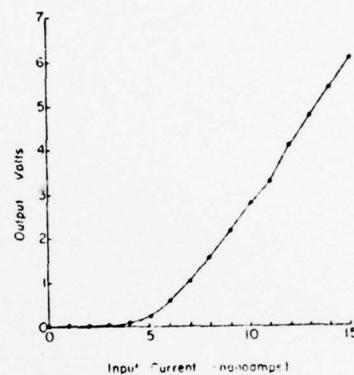


Fig. 2. The current to voltage response curve of the telemetry system.

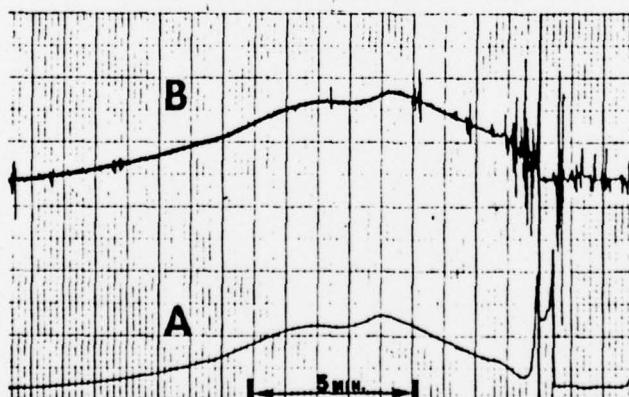


Fig. 3. Sample data taken from electrodes implanted in rabbit striated muscle. Curve A: The input current to the transmitter. Curve B: The output voltage of the telemetry system.

monitoring requires restrained or anesthetized animal conditions if hard wire methods are used. The use of a telemetry system is a practical alternative in both instances.

One of the reasons for developing a telemetry system for use with the electrode package described was that previous experiments using restrained unanesthetized rabbits yielded data that could have been influenced by the restraining stress. Using the telemetry system permitted animal mobility, eliminating the possible restraining influence. However, in this experiment, no significant difference was found between data obtained for the restrained and unrestrained animal and, in both cases, there was no response to changes in respiratory oxygen or carbon dioxide concentrations. Therefore, in order to adequately demonstrate the applicability of the telemetry system, an anesthetized animal was required.

There were a number of features about the telemetry system that need improvement. The main problems encountered were instability of the transmitter frequency due to varying stray capacitance antenna and receiver drift due to the pulsed FM nature of the transmitter. It would also be beneficial to reduce the size of the overall package.

In conclusion, while our present system needs additional work on further miniaturization and other technical features, we have demonstrated the feasibility of monitoring oxygen tension from biological subjects. Results of this study show that it is possible to transmit data from chronically implanted oxygen electrodes. It permits unrestrained, unanesthetized preparations to be studied, with local oxygen tensions being monitored under the influence of many types of forcing agents.

ACKNOWLEDGEMENTS

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